Seizure 1995; 4: 249–258

REVIEW ARTICLE

Axonal sprouting and synaptogenesis in temporal lobe epilepsy: possible pathogenetic and therapeutic roles of neurite growth inhibitory factors

ANDREW J. LARNER

University of Cambridge Department of Anatomy, Downing Street, Cambridge CB2 3DY, University Department of Neurology, Addenbrooke's Hospital, Cambridge CB2 2QQ, and Gonville & Caius College, Trinity Street, Cambridge CB2 1TA, UK

Correspondence to: Dr A.J. Larner, Department of Anatomy, Downing Street, Cambridge CB2 3DY, UK

Axonal sprouting and synaptic reorganization within the temporal lobe following neuronal injury have been implicated in the pathogenesis of temporal lobe epilepsy (TLE). The molecular species responsible for these structural changes have yet to be fully defined. The recent characterization of molecules whose normal function within the nervous system is to inhibit neurite outgrowth and synaptogenesis prompts the suggestion that a diminution or loss of such molecules might be of relevance to the pathogenesis of TLE. If so, the possibility of developing a novel therapeutic approach to TLE, distinct from currently available symptomatic therapies, to arrest the pathogenetic processes is also raised.

Key words: temporal lobe epilepsy; neurite growth-inhibitory factors; axonal sprouting; synaptogenesis; Alzheimer's disease.

INTRODUCTION

Approximately 40% of all cases of epilepsy take the form of complex partial seizures (CPS)[1], the majority of which originate within the temporal lobe. Patients with this epileptic syndrome have a less favourable prognosis for seizure control compared to those with generalized seizures, 30% of the former remaining intractable to symptomatic medical therapy[2]. Although a larger number of such patients than heretofore may benefit from surgical intervention, the statistics nonetheless suggest that there remains a need for novel therapeutic modalities to control or prevent the development of complex partial seizures of temporal lobe origin.

Epileptic phenomena are characterized by neuronal hyperexcitability and paroxysmal discharge of cortical neurones. Neurophysiological studies in both animal models and man suggest that such excessive and abnormally synchronous firing of neurones results from a concurrent enhancement of excitatory synaptic transmission and a decrease in inhibitory synaptic transmission, the resulting imbalance leading to neuronal hyperexcitability[3]. These changes in synaptic transmission are mediated by the neurotransmitters glutamate and γ-aminobutyric acid (GABA), respectively[3].

Despite this understanding of the neurophysiology and neurochemistry of the epileptic temporal lobe, relatively little is currently known about underlying neuroanatomical changes. The commonest neuropathological change, seen in the temporal lobes of around 50% of individuals with complex partial seizures[4], is variously known as Ammon's horn sclerosis (AHS), incisural sclerosis, mesial temporal sclerosis or hippocampal sclerosis.

1059–1311/95/040249+10 $12·00/0

© 1995 British Epilepsy Association

Macroscopical features include fibrous gliosis, shrinkage and atrophy, whilst microscopically there is a patterned neuronal loss affecting principally the pyramidal cell layers in the hippocampal subfields CA1, CA3, and in the dentate hilus, but sparing the granule cells of the dentate gyrus (or fascia dentata) and some pyramidal cells in hippocampal region CA2[4,5] (hippocampal nomenclature of Lorente de No[6]). In addition to cell loss, neuronal impregnation studies using the Golgi technique have shown dendritic changes in the epileptic hippocampus[7]. DeLorenzo and Glaser observed a dramatic reorganization of both dendritic and axonal projections from pyramidal and other neurones within the hippocampus, suggesting the formation of new neuronal processes and synaptic contacts in addition to neuronal loss in the hippocampus[8]. Hence, they advanced the hypothesis that reactive neuronal plasticity, occurring in response to neuronal injury and degeneration, might play a role in the development and persistence of the epileptic state[8,9].

SPROUTING AND NEURONAL PLASTICITY

In the vertebrate peripheral nervous system, injured axons have the capacity to regenerate by sprouting from intact nodes of Ranvier (nodal sprouting) or from the terminals of injured axons themselves (terminal sprouting). Axonal elongation through remaining Schwann cell tubes permits reconstitution of appropriate synaptic contacts (homotypic sprouting) and full restoration of function. Undamaged axons can also sprout collateral branches in response to partial deafferentation, and these collateral sprouts are able to fill vacated synaptic contacts (heterotypic sprouting) thus reorganizing synaptic circuitry[10]. This neuronal plasticity probably underlies the ability of many sub-mammalian vertebrates to achieve functional regeneration following central nervous system (CNS) injury[11], and may be the physiological correlate of learning and memory.

In contrast to the peripheral nervous system, damaged axons in the mammalian CNS usually fail to regenerate, principally because the mammalian CNS environment is inimical to axonal elongation following injury[11]. Collateral sprouting does occur, but axon elongation to re-establish appropriate synaptic targets is often not possible because of the non-permissive nature of the CNS environment. Aberrant, heterotypic, local synapses may, however, be formed. This synaptic reorganization may account for the limited functional recovery seen after mammalian CNS injury, but it may also have less beneficial consequences, for example the development of spasticity is often ascribed to heterotypic sprouting. Sprouting is thus a ubiquitous feature of neuronal injury, whose functional consequences may be beneficial, neutral or detrimental, depending on the precise situation in which it occurs.

NEURITE GROWTH-INHIBITORY FACTORS

The paucity of regeneration following injury to the mammalian CNS as compared to sub-mammalian vertebrates has been chiefly ascribed to the constitutively non-permissive nature of the mammalian CNS environment for axonal regeneration. It is probable that this non-permissive environment is due, at least in part, to the presence of cell-surface and diffusible molecules which inhibit nerve outgrowth[12,13]. A number of nerve growth-inhibitory factors (NGIFs) have been described recently, some of which have been partially characterized at the molecular level (Table 1). As the description of NGIFs is in its infancy, more candidate NGIFs are likely to be discovered in the future.

Schwab and his colleagues have identified two proteins associated with oligodendrocytes and myelin in mammalian CNS which inhibit neurite outgrowth[14] and cause collapse of

Table 1: Candidate neurite growth-inhibitory molecules

Source	Name	Reference
Chick posterior half somite	—	20
Chick posterior optic tectum	—	21
Chick brain	Collapsin	22
Various	CSPG	12
Astrocytes	Cytotactin	12
Mammalian myelin	NI35, NI250	14
Human astrocytes	GIF	24
?	H-sema III	23
Mammalian myelin	MAG	12
Various	T-cadherin	12

Abbreviations: GIF, Growth Inhibitory Factor; CSPG, Chondroitin Sulphate Proteoglycan; MAG, Myelin Associated Glycoprotein.

neuronal growth cones *in vitro*[15]. These proteins are expressed at lower levels in the CNS of fish, in which spontaneous regeneration does occur, but are not up-regulated by injury as in the mammalian CNS[16]. Antibodies to these myelin-associated inhibitors allow more extensive axonal regeneration following CNS lesions in mammals[17] and more extensive collateral sprouting into denervated areas[18].

Studies of developmental phenomena have provided further examples of neuronal growth-inhibitory factors, which are thought to play important roles in axonal guidance[19]. As motor and sensory axons first emerge from the spinal cord they are confined to the anterior half of the adjacent paraxial mesoderm of the somite due to the presence in the posterior half-somite of a growth-inhibitory glycoprotein which causes growth cone collapse *in vitro*[20]. The precise patterning of axons arriving in the posterior and anterior tectum from the nasal and temporal axons only[21]. Chondroitin sulphate proteoglycans (CSPGs) are also candidate repellent molecules, having been associated with neurite avoidance behaviour in a number of systems, as have other extracellular matrix glycoproteins such as cytotactin (tenascin)[12]. with neurite avoidance behaviour in a number of systems, as have other extracellular matrix glycoproteins such as cytotactin (tenascin)[12].

Raper and his colleagues have recently characterized a protein from embryonic and adult chick brain which induces paralysis and collapse of growth cones *in vitro*[22]. Although its *in-vivo* functions are not definitely known, collapsin is homologous to fasciclin IV, an axon guidance molecule found in grasshoppers. That such proteins may be of general relevance was indicated by the finding of genes encoding similar proteins in *Drosophila*, grasshopper, chick and human, designated the 'semaphorin' family[23]. The human homologue, H-sema III, has not yet been isolated, nor has its functional activity been defined.

It is probable that a number of classes of neuro-inhibitory molecules exist in vertebrates. A 68-amino acid protein of astrocytic origin which can inhibit neurite outgrowth (GIF) has been characterized from normal human brain[24,25]. The possibility that derangement of NGIFs might be involved in the pathogenesis of certain diseases of the nervous system is suggested by the observation that GIF is deficient in Alzheimer's disease brain[24], in which a cortical sprouting response is characteristically seen.

AXONAL SPROUTING AND SYNAPTIC REORGANIZATION IN TLE

Evidence to support the hypothesis that neuronal plasticity underlies the development and persistence of TLE[9] has been forthcoming from both animal models and clinical material.

The granule cells within the dentate gyrus normally project their axons, the mossy fibres, to the giant pyramidal neurones of the adjacent CA3 subfield of the hippocampus[6]. Lesion studies have shown that following damage to pyramidal cells in CA3, mossy fibres may sprout axon collaterals into the inner molecular layer of the dentate gyrus where the dendrites of granule cells are normally situated[26,27]. Such a recurrent pathway could theoretically lead to abnormal excitatory input to dentate granule cells ('feedback excitation'), and hence constitute a plausible anatomical substrate for the development of repetitive electrical discharge, neuronal hyperexcitability and epileptic seizures.

This sprouting response may be demonstrated using various markers for mossy fibres and their axon collaterals. The high zinc content of mossy fibres permits their visualization by Timm-sulphide histochemistry[28]. Mossy fibres are excitatory, and high densities of excitatory amino acid receptors are found in regions to which they project (for example CA3), particularly in their terminal arborization. Kainic acid (KA) binding sites are located presynaptically on mossy fibre terminals, and hence an increased density of high affinity KA binding sites is also a marker of mossy fibre sprouting. Immunohistochemistry for dynorphin A, an opioid peptide contained and released by mossy fibres and which can generate epileptiform activity in the CA3 region, may also be used to demonstrate sprouting. These techniques have been used to provide evidence indicative of sprouting within the epileptic, as well as the experimentally lesioned, hippocampus.

ANIMAL MODELS: KAINIC ACID AND KINDLING

The discovery that administration of the potent limbic convulsant kainic acid (KA) to rats produces lesions remarkably similar to human mesial temporal sclerosis has provided a useful model system for the study of TLE[29]. After KA-induced lesions, axons of the dentate

granule cells (mossy fibres) are observed to sprout collateral fibres which grow across the granule cell layer to form a plexus in the inner molecular layer of the granule cell dendrites[30]. Although this recurrent pathway may seem a plausible and attractive neuroanatomical explanation for seizure activity, the exact relation of structural change to functional consequence remains a critical question. Although evidence has been presented suggesting that these synaptic rearrangements do promote seizure activity[30,31] the case is not entirely proven. Changes are unlikely to be confined solely to the mossy fibres: other neuronal systems are also likely to sprout, for example GABAergic fibres[32], suggesting that anomalous inhibitory synapses, as well as excitatory synapses may contribute to hyperexcitability. Indeed, Sloviter has advanced evidence suggesting that hyperexcitability precedes dentate synaptic reorganization, and is associated with a selective loss of interneurones which is presumably the stimulus for axon sprouting[33]. According to this interpretation, mossy fibre sprouts innervate the GABAergic basket cells of Cajal, the most important type of interneurone in the hippocampus, so enhancing the inhibition of granule cells, thus producing a primarily inhibitory, and hence beneficial, rather than epileptogenic, effect. The observations of Cronin *et al* go some way to reconciling the disparate viewpoints: their observations that $GABA_A$ receptor antagonists produce synchronous bursts in granule cells only in hippocampal slices which show sprouting suggests that both recurrent inhibitory and excitatory circuits are formed, the latter emerging only when GABA-mediated synaptic inhibition is lost[34].

Similar morphological observations have been made in a further model of TLE, namely the kindled rat[35]. Administration of repeated subconvulsive electrical or chemical stimuli to the hippocampal afferent pathways eventually produces seizures of increasing intensity and a permanent susceptibility to seizures, the kindled state. Such altered functional activity in the hippocampus results in morphological sprouting of mossy fibres into the supragranular zone of the dentate gyrus as evidenced by Timm histochemistry[36], presumably as a result of altered gene expression (*vide infra*). Moreover, the initial appearance of mossy fibre synaptic terminals in the supragranular layer occurs four days after the initiation of kindling, a time course compatible with axon sprouting[37]. Aberrant Timm staining, reflecting axon sprouting, progresses in parallel with kindling and persists for up to eight months after the last kindled seizure. Kindling also results in progressive neuronal loss within the hippocampus[38]. However, the functional significance of these structural changes, as in the KA model, remains to be conclusively defined.

Table 2: Studies of sprouting in human tissue

Markers of sprouting	Reference
(A) Mossy fibre sprouting	
KA autoradiography	45
Dynorphin A immunoreactivity	48
Timm histochemistry	41
Timm histochemistry and dynorphin A immunoreactivity	42
Timm histochemistry	40
KA/NMDA autoradiography	46
Timm histochemistry	43
Timm histochemistry	44
(B) Other systems investigated	
Immunohistochemistry for GABA, somatostatin, neuropeptide Y	48
Immunohistochemistry for glutamate decarboxylase (GAD)	50
Immunohistochemistry for peptides: somatostatin, CCK, VIP, dynorphin A	49
$GABA_A$, KA, NMDA receptor autoradiography	47
(C) Functional Studies	
Orthodromic stimulation via perforant path	51
Abnormal responses to NMDA	52
Antidromic stimulation of mossy fibres	44

HUMAN STUDIES

It has been pointed out that experiments in animal models of epilepsy show mechanisms of models, which cannot necessarily be extrapolated to human epilepsy[39]. It is therefore important that, despite the difficulties in obtaining adequate control tissues, studies of human epileptic hippocampus be undertaken to determine whether similar anatomical changes occur and what their functional significance might be[35,40].

Studies of human hippocampal tissue recovered either *post mortem* or *ante mortem* (temporal lobes surgically resected for intractable epilepsy) have demonstrated changes indicative of axonal sprouting and synaptic rearrangement in epileptic tissue (summarized in Table 2). Increased Timm staining in the supragranular layer of the dentate gyrus has been reported by several groups in both adult[41–44] and childhood epilepsy[40]. These

qualitative results from Timm histochemistry have been complemented by quantitative autoradiographic studies of excitatory amino acid binding sites. An increased density of KA receptors was observed in the CA3 region and the fascia dentata by Represa *et al*[45], whilst Geddes *et al*[46] found an increase in both KA and N-methyl-D-aspartate (NMDA) receptors in the entorhinal cortex (the source of afferent input to the dentate gyrus via the perforant pathway) and the dentate gyrus. The latter observation was confirmed by McDonald *et al*[47]. Increased immunoreactivity for dynorphin A has been reported to closely parallel the changes in Timm staining[42,48].

As with animal models, mossy fibre sprouting is unlikely to be the only change in the human epileptic hippocampus. Immunohistochemical studies have shown loss of the peptides somatostatin and neuropeptide Y, suggesting a loss of inhibitory interneurone connections[48]. Loss of somatostatin producing interneurones with up-regulation of dentate somatostatin receptors has been reported to be a specific and characteristic change in human TLE, since levels of other peptides (CCK octapeptide, VIP, dynorphin A1-17) remain normal[49]. Normal immunoreactivities for GABA[48] and for glutamate decarboxylase, the GABA synthetic enzyme[50], have been reported. Despite this anatomical evidence for preservation of the GABAergic system, this does not necessarily imply functional normality. The changes are similar to those seen in the KA model system where loss of GABA-mediated inhibition is thought to occur as a result of synaptic rearrangement of GABAergic neurones[34]; there is some evidence for similar pathophysiology in human epileptic tissue[51] (*vide infra*), perhaps secondary to loss of interneurones. Furthermore, $GABA_A$ receptors are decreased in certain hippocampal areas[47].

As with animal studies, the critical question of the functional significance of these morphological changes in human hippocampus is uncertain. Suggestive circumstantial evidence includes the correlation between surgical removal of 'sprouted' hippocampus and reduced seizure activity[41]. More direct evidence is forthcoming from the recording of electrical responses from dentate granule cells in resected temporal lobes following either orthodromic (perforant path) or antidromic (mossy fibre axons and their collaterals) stimulation. Isokawa and Levesque correlated epileptogenic responses of individual dentate granule cells to applications of NMDA with loss of dendritic spines and beading of dendritic shafts[52]. These changes may be the functional correlate of the dendritic abnormalities originally observed by Scheibel *et al*.[7] These hyperexcitable responses of dentate granule cells may also reflect a decrease in GABA-mediated synaptic inhibition[51], since similar responses in the normal rat hippocampus could only be elicited in the presence of the $GABA_A$ antagonist bicuculline. The correlation of abnormal antidromic field responses with extent of mossy fibre reorganization (as judged by degree of Timm staining) in a subgroup of TLE patients supports the hypothesis that mossy fibre terminals in the supragranular layers do have functional consequences which may underpin seizure activity, at least in some patients[44].

Hence, human studies have borne out the animal models which suggest an epileptogenic role for axonal sprouting and synaptic reorganization in the temporal lobe.

MOLECULAR FACTORS INVOLVED IN SPROUTING

The molecular factors which induce and modulate dentate granule cell mossy fibre sprouting in TLE are currently unknown, but some circumstantial experimental evidence has been forthcoming.

The development, survival and maintenance of neuronal cells is dependent on retrogradely-transported neurotrophic factors, present in limiting amounts in target areas. Up-regulation of such neurotrophic factors might prompt axonal sprouting. A number of experimental studies have now shown rapid (within hours) and selective, but transient (24–48 hours duration), increases in the expression of genes for the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in dentate granule cells after seizures[53–55], but neurotrophin-3 (NT-3) mRNA levels fall[54,55]. The largest alterations are in BDNF mRNA levels, and these elevations are also seen in areas outside the hippocampus[53]. mRNAs for the neurotrophin receptors *trkB* and *trkC* also rise in dentate granule cells, but *trkA* is unaltered[55].

Lowenstein and colleagues have documented an increase in neurotrophic activity in saline extracts of homogenized temporal lobe from KA-treated rats using a neuronal survival

assay[56]. This activity was significantly increased 12 hours after kainate treatment, peaked after seven days, but was still evident after two months. Antibody-neutralization assays at seven days suggested that at least part of this activity consisted of NGF, most of the remainder being ciliary neurotrophic factor (CNTF). NGF-like activity has also been detected in fresh human cortical tissue (resected temporal lobe seizure foci), which can be significantly inhibited in a bioassay of sympathetic neurite outgrowth by polyclonal antiserum to NGF[57]. The difficulty in obtaining suitable control tissues makes it impossible to adjudge whether this activity is elevated.

Immunoreactivity for molecules related to the neural cell-adhesion molecule (NCAM), which promotes neurite outgrowth, has been found to correlate both spatially and temporally with the pattern of Timm staining in the KA-treated epileptic rat[58]. At the electron microscopic level, antibody to NCAM-like activity stains both axonal profiles and astrocytes. Hence NCAM may contribute to the synaptic rearrangement of mossy fibres seen in TLE, as may other adhesion or substrate proteins such as L1[58]. The detection of increased levels of mRNA for the axon growth-associated protein GAP-43 in the granule cells of KA-treated rats is also consistent with sprouting of mossy fibres[59].

The up-regulation of genes expressing the neurotrophins NGF and BDNF after experimental hippocampal neuronal injury is brief, lasting a maximum of 48 hours, and the peak of the neurotrophic activity in hippocampal extracts, ascribed mainly to NGF, occurs seven days after injury. Although such changes might be adequate to initiate a sprouting response in the injured hippocampus, it is not clear whether they would be sufficient to maintain it for a period of months in experimental animals[37] or years in man.

A PATHOGENETIC ROLE FOR NGIFs IN TLE?

The ontogeny of neuronal pathfinding may depend on the differential expression of factors which are both attractive to and repulsive for neuronal growth cones[19]. This differential expression may be recapitulated in pathological states. Hence, a further, intriguing, possibility is that axonal sprouting in TLE may result not only from increased levels of neurotrophins and adhesion molecules of the cell surface and extracellular matrix, but also from a diminution in neurite growth-inhibitory factors (NGIFs). The constitutively non-permissive nature of the mature mammalian CNS to axonal growth may suggest that a loss of inhibitory (NGIF) activity is more important for sustained axonal growth and synaptic reorganization than a transient increase in neurotrophin expression. An important precedent for such a suggestion stems from studies of tissue extracts from Alzheimer's diseased (AD) brains: AD brain is characterized pathologically by a sprouting response of partially deafferented neurones throughout the cortical neuropil. Using a neuronal survival assay, Uchida *et al* initially described an increase in neurotrophic activity in AD brain[60], but this eventually proved to be a loss of a neurite growth-inhibitory protein of astrocytic origin[24,25]. Furthermore, Schwab and Thoenen have presented evidence that adult CNS inhibitory activity cannot be overcome by the stimulatory effects of neurotrophic factors[61]. Preliminary experiments using an assay of neuronal growth cone collapse, a standard assay for detection of neurite growth-inhibitory molecules[62], have demonstrated abundant collapsing activity in detergent extracts of homogenized rat hippocampus, but no obvious diminution in specific activity in hippocampi seven days after intraperitoneal kainic acid, although longer time points have yet to be tested (Larner, A.J., Johnson, A.R., Cook, G.M.W., Rudge, J.S., Keynes, R.J., unpublished observations).

The molecular probes to confirm or refute this speculation are only now becoming available. For example, it will be interesting to learn whether there is increased expression of GAP-43 in human epileptic hippocampus, as in the KA rat model[59], since increased expression of GAP-43 may correlate with loss of myelin-associated NGIFs[63].

A THERAPEUTIC ROLE FOR NGIFs IN TLE?

Just as an increased understanding of the neurophysiology and neurochemistry of epilepsy has led to the rational development of new antiepileptic drugs[64], the new insights into the neuroanatomical basis of epilepsy could also herald the development of a new class of reagents for the treatment of epilepsy.

If aberrant neuronal growth and reorganization of synaptic circuitry is central to the

pathogenesis of TLE, then therapy with agents whose normal function is to inhibit nerve growth and synaptogenesis would seem logical, the more so if endogenous NGIF deficiency is one of the key molecular determinants of the pathogenetic cascade. Data have emerged to suggest that the myelin-associated inhibitors characterized by Schwab and his colleagues act in the unlesioned CNS to suppress sprouting and maintain synaptic stability[18]. Furthermore, administration of antibodies to these inhibitors is followed by an expanded pattern of collateral sprouting into a previously denervated area[63], suggesting that the normal role of these NGIFs may be to restrict postnatal plasticity. Hence, exogenous NGIFs and their analogues might be used to diminish or prevent sprouting and so arrest the pathogenetic process.

Studies on a potential therapeutic role for NGIFs in TLE will be facilitated by the availability of two animal models of the condition, namely kainic acid treated and kindled rats[35]. Since the time course of axon sprouting has been carefully documented in the kindled rat[37], it should be feasible to make correlations between time of administration of NGIF therapy and efficacy in reducing seizures. Intuitively, one might imagine that the greatest benefit of NGIFs will be as prophylactic agents, preventing or diminishing axon sprouting immediately after injury to hippocampal neurons. The documented latent period between a hippocampal neuronal insult and the onset of axonal sprouting may represent a window of therapeutic opportunity for the prophylactic use of NGIFs[37]. Alternatively, the possibility that a long-lasting diminution in the level of NGIFs permits axonal sprouting in the hippocampus (*vide supra*) suggests that exogenous NGIF treatment long after the original injury may also be of therapeutic value.

Could NGIFs have a prophylactic and/or therapeutic role in human TLE? It is well recognized that AHS, the commonest neuropathological correlate of TLE, is significantly associated with a history of febrile convulsions, birth injury, and episodes of status epilepticus[4]. These phenomena might be indicators for NGIF therapy, but would also encompass many individuals who might develop AHS but were not destined to subsequently develop TLE. It is recognized that although initial neuronal damage in the hippocampus is linked closely to metabolic and ionic events arising during the greatly enhanced neuronal activity of a seizure, the consequent glial reaction and scarring gradually 'ripen'[65]. At some later stage these changes may disrupt normal cerebral activity with the induction of further epileptic attacks. This 'ripening' period may be analogous to the window of therapeutic opportunity in animal models[37], but its exact duration remains to be defined. The observation of dendritic changes in the human epileptic hippocampus also suggests that TLE might reflect an ongoing pathological process[7] rather than resulting from a single, discrete, cortical insult early in life as was once thought. Such observations further suggest a place for NGIF therapy even many years after the onset of seizures. Gowers recognized, almost a century ago, that epilepsy is a self-perpetuating disease, the abnormal functional activity of each seizure occurring in such a way as to facilitate the subsequent recurrence of the same functional activity[66]. Loss of NGIFs may be the physiological correlate of this self-perpetuating activity, and hence exogenous NGIF therapy may represent a mechanism to abrogate this residual effect.

Which of the NGIFs described might be used in a therapeutic trial? Currently, this is an academic question since only two NGIFs have been defined at both the protein and genetic level to date, namely human metallothionein-like GIF[24] and chick collapsin[22]. Both of these molecules, and some of the other NGIFs which are as yet only partially characterized, are known to be glycoproteins, for example the posterior half somite-derived protein[20] and the posterior tectal membrane protein[21]. Proteins will not cross the intact blood–brain barrier, so obviating the simple approach of intravascular injection or infusion therapy and necessitating a more invasive approach, namely intracerebral or intraventricular injection. Such a requirement would severely limit the clinical acceptability of NGIF treatment. Linkage to a lipid-soluble carrier molecule might circumvent this problem.

The definition of NGIFs at the protein and genetic level[22–25] should be the prelude to the characterization of the cellular receptor(s) for these proteins. This will be an advance of fundamental importance with many implications. It will permit the development of so-called 'small molecule' ligands, analogues of NGIFs, which, unlike proteins, could cross the blood–brain barrier following intravascular administration. Such reagents would provide not only new therapeutic agents, but also the possibility

of functional imaging of axonal sprouting in an injured temporal lobe by conjugation of ligands with positron emitting isotopes; imaging of functional rearrangement after brain injury has already proven possible by this method[67]. Functional imaging should improve the selection of potential candidates for NGIF therapy by identifying those in whom sprouting is actually occurring (pathogenetic heterogeneity in TLE is certainly possible[44]), and would also indicate the distribution and localization of administered ligands within the CNS.

Kindling, and by extension sprouting, is thought to be relevant to the psychopathology of TLE as well as epileptogenesis[68], and manipulation of various neurotransmitter receptor types by drug therapy has occasionally resulted in unwanted psychotic effects. NGIF therapy, aimed at a neuroanatomical rather than a neurochemical target, should hopefully avoid this pitfall.

CONCLUSION

The study of axonal sprouting and synaptic reorganization in the epileptic hippocampus has given new insights into the pathogenesis of temporal lobe epilepsy. Basic research studies have characterized molecules which normally function within the CNS to inhibit neurite outgrowth and synapse formation. These findings have prompted the suggestion of both a possible pathogenetic role for such molecules in temporal lobe epilepsy, and also the possible development of novel therapeutic approaches utilizing these molecules which aim to diminish or prevent the pathogenetic, epileptogenic changes. Although still a long way from routine clinical application, these studies may eventually enable physicians to tackle the pathological anatomy of epilepsy, which is recognized to reinforce itself with each successive seizure.

ACKNOWLEDGEMENTS

Thanks to Dr G.M.W. Cook for constructive criticism of the manuscript. This article is a revised version of an essay which was highly commended by the judges of the Gowers Prize (Young Physician) Competition of the International League Against Epilepsy 1994, and of the winning entry in the British Society for Clinical Neurophysiology Essay Prize 1994.

REFERENCES

1. Hauser, W.A. and Kurland, L.T. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia* 1975; **16**: 1–66.
2. Willmore, L.J. Epilepsy. In: *Prognosis of Neurological Disorders* (Eds R.W. Evans, D.S. Baskin and F.M. Yatsu). New York, Oxford University Press, 1992: pp. 483–496.
3. Jefferys, J.G.R. Experimental neurobiology of epilepsies. *Current Opinion in Neurology and Neurosurgery* 1994; **7**: 113–122.
4. Bruton, C.J. *The Neuropathology of Temporal Lobe Epilepsy*. Oxford, Oxford University Press, 1988.
5. Margerison, J.H. and Corsellis, J.A.N. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain* 1966; **89**: 499–530.
6. Lorente de No, R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *Journal of Psychology and Neurology* 1934; **46**: 113–177.
7. Scheibel, M.E., Crandall, P.H. and Scheibel, A.B. The hippocampal-dentate complex in temporal lobe epilepsy. A Golgi study. *Epilepsia* 1974; **15**: 55–80.
8. DeLorenzo, R.J. and Glaser, G.H. Neuropathologic changes and neuronal plasticity in temporal lobe-limbic epilepsy. *Neurology* 1981; **31** (Number 4, Part 2): 114.
9. DeLorenzo, R.J., Glaser, G.H., Delucia, P. and Schwartz, D. The role of neuronal plasticity in epilepsy. *Neurology* 1982; **32**: A92.
10. Brown, M.C., Hopkins, W.G. and Keynes, R.J. *Essentials of Neural Development*. Cambridge, Cambridge University Press, 1991 (2nd edit.): pp. 132–135.
11. Larner, A.J., Johnson, A.R. and Keynes, R.J. Regeneration in the vertebrate nervous system: phylogeny, ontogeny, and mechanisms. *Biological Reviews* 1995: (in press).
12. Keynes, R.J. and Cook, G.M.W. Repulsive, inhibitory, and stop signals. *Current Opinion in Neurobiology* 1995; **5**: (in press).
13. Schwab, M.E., Kapfhammer, J.P. and Bandtlow, C.E. Inhibitors of neurite growth. *Annual Review of Neuroscience* 1993; **16**: 565–595.
14. Caroni, P. and Schwab, M.E. Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. *Journal of Cell Biology* 1988; **106**: 1281–1288.
15. Bandtlow, C.E., Schmidt, M.F., Hassinger, T.D., Schwab, M.E. and Kater, S.B. Role of intracellular calcium in NI-35-evoked collapse of neuronal growth cones. *Science* 1993; **259**: 80–83.
16. Sivron, T. and Schwartz, M. The enigma of myelin-associated growth inhibitors in spontaneously regenerating nervous systems. *Trends in Neurosciences* 1994; **17**: 277–281.
17. Schnell, L. and Schwab, M.E. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* 1990; **343**: 269–272.

18. Kapfhammer, J.P., Schwab, M.E. and Schneider, G.E. Antibody neutralization of neurite growth inhibitors from oligodendrocytes results in expanded pattern of postnatally sprouting retinocollicular axons. *Journal of Neuroscience* 1992; **12**: 2112–2119.
19. Pini, A. Growth cones say no. *Current Biology* 1994; **4**: 131–133.
20. Davies, J.A., Cook, G.M.W., Stern, C.D. and Keynes, R.J. Isolation from chick somites of a glycoprotein fraction that causes collapse of dorsal root ganglion growth cones. *Neuron* 1990; **4**: 11–20.
21. Stahl, B., Muller, B., Von Boxberg, Y., Cox, E.C. and Bonhoeffer, F. Biochemical characterization of a putative axonal guidance molecule of the chick visual system. *Neuron* 1990; **5**: 733–743.
22. Luo, Y., Raible, D. and Raper, J.A. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* 1993; **75**: 217–227.
23. Kolodkin, A.L., Matthes, D.J. and Goodman, C.S. The *semaphorin* genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell* 1993; **75**: 1389–1399.
24. Uchida, Y., Takio, K., Titani, K., Ihara, Y. and Tomonaga, M. The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron* 1991; **7**: 337–347.
25. Tsuji, S., Kobayashi, H., Uchida, Y., Ihara, Y. and Miyatake, T. Molecular cloning of human growth inhibitory factor cDNA and its down regulation in Alzheimer's disease. *EMBO Journal* 1992; **11**: 4843–4850.
26. Laurberg, S. and Zimmer, J. Lesion-induced sprouting of hippocampal mossy fiber collaterals to the fascia dentata in developing and adult rats. *Journal of Comparative Neurology* 1981; **200**: 433–459.
27. Frotshcer, M. and Zimmer, J. Lesion-induced mossy fibers to the inner molecular layer of the rat fascia dentata: identification of postsynaptic granule cells by the Golgi-EM technique. *Journal of Comparative Neurology* 1983; **215**: 299–311.
28. Danscher, G. Histochemical demonstration of heavy metals: a revised version of the silver sulphide method suitable for both light and electron microscopy. *Histochemistry* 1981; **71**: 1–16.
29. Nadler, J.V. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sciences* 1981; **29**: 2031–2042.
30. Tauck, D.L. and Nadler, J.V. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *Journal of Neuroscience* 1985; **5**: 1016–1022.
31. Cronin, J. and Dudek, F.E. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. *Brain Research* 1988; **474**: 181–184.
32. Davenport, C.J., Brown, W.J. and Babb, T.L. Sprouting of GABAergic and mossy fiber axons in dentate gyrus following intrahippocampal kainate in the rat. *Experimental Neurology* 1990; **109**: 180–190.
33. Sloviter, R.S. Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. *Neuroscience Letters* 1992; **137**: 91–96.
34. Cronin, J., Obenaus, A., Houser, C.R. and Dudek, F.E. Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. *Brain Research* 1992; **573**: 305–310.
35. Sutula, T.P. Reactive changes in epilepsy: cell death and axon sprouting induced by kindling. *Epilepsy Research* 1991; **10**: 62–70.
36. Sutula, T.P., Xiao-Xian, H., Cavazos, J. and Scott, G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* 1988; **239**: 1147–1150.
37. Cavazos, J.E., Golarai, G. and Sutula, T.P. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *Journal of Neuroscience* 1991; **11**: 2795–2803.
38. Cavazos, J.E., Das, I. and Sutula, T.P. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *Journal of Neuroscience* 1994; **14**: 3106–3121.
39. Fisher, R.S. Neuronal damage and epilepsy: basic and clinical interface. *Epilepsy Research* 1991; **10**: 80–89.
40. Ben-Ari, Y. and Represa, A. Brief seizure episodes induce long-term potentiation and mossy fibre sprouting in the hippocampus. *Trends in Neurosciences* 1990; **13**: 312–318.
41. Sutula, T.P., Cascino, G., Cavazos, J., Parada, I. and Ramirez, L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Annals of Neurology* 1989; **26**: 321–330.
42. Houser, C.R., Miyashiro, J.E., Swartz, B.E., Walsh, B.O., Rich, J.R. and Delgado-Escueta, A.V. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *Journal of Neuroscience* 1990; **10**: 267–282.
43. Babb, T.L., Kupfer, W.R., Pretorius, J.K., Crandall, P.H. and Levesque, M.F. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience* 1991; **42**: 351–363.
44. Masukawa, L.M., Urano, K., Sperling, M., O'Connor, M.J. and Burdette, L.J. The functional relationship between antidromically evoked field responses of the dentate gyrus and mossy fiber reorganization in temporal lobe epileptic patients. *Brain Research* 1992; **579**: 119–127.
45. Represa, A., Robain, O., Tremblay, E. and Ben-Ari, Y. Hippocampal plasticity in childhood epilepsy. *Neuroscience Letters* 1989; **99**: 351–355.
46. Geddes, J.W., Cahan, L.D., Cooper, S.M., Kim, R.C., Choi, B.H. and Cotman, C.W. Altered distribution of excitatory amino acid receptors in temporal lobe epilepsy. *Experimental Neurology* 1990; **108**: 214–220.
47. McDonald, J.W., Garofalo, E.A., Hood, T. *et al.* Altered excitatory and inhibitory amino acid receptor binding in hippocampus of patients with temporal lobe epilepsy. *Annals of Neurology* 1991; **29**: 529–541.
48. de Lanerolle, N.C., Kim, J.H., Robbins, R.J. and Spencer, D.D. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Research* 1989; **495**: 387–395.
49. Robbins, R.J., Brines, M.L., Kim, J.H. *et al.* A selective loss of somatostatin in the hippocampus of patients with temporal lobe epilepsy. *Annals of Neurology* 1991; **29**: 325–332.
50. Babb, T.L., Pretorius, J.K., Kupfer, W.R. and Crandall, P.H. Glutamate decarboxylase immunoreactive neurons are preserved in human epileptic hippocampus. *Journal of Neuroscience* 1989; **9**: 2562–2574.
51. Masukawa, L.M., Higashima, M., Kim, J.H. and Spencer, D.D. Epileptiform discharges evoked in hippocampal brain slices from epileptic patients. *Brain Research* 1989; **493**: 168–174.
52. Isokawa, M. and Levesque, M.F. Increased NMDA re-

sponses and dendritic degeneration in human epileptic hippocampal neurons in slices. *Neuroscience Letters* 1991; **132**: 212–216.
53. Isackson, P.J., Huntsman, M.M., Murray, K.D. and Gall, C.M. BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. *Neuron* 1991; **6**: 937–948.
54. Rocamora, N., Palacios, J.M. and Mengod, G. Limbic seizures induce a differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3, in the rat hippocampus. *Molecular Brain Research* 1992; **13**: 27–33.
55. Bengzon, J., Kokaia, Z., Ernfors, P. *et al.* Regulation of neurotrophin and *trkA*, *trkB* and *trkC* tyrosine kinase receptor messenger RNA in kindling. *Neuroscience* 1993; **53**: 433–446.
56. Lowenstein, D.H., Seren, M.S. and Longo, F.M. Prolonged increases in neurotrophic activity associated with kainate-induced hippocampal synaptic reorganization. *Neuroscience* 1993; **56**: 597–604.
57. Crutcher, K.A., Scott, S.A., Liang, S., Everson, W.V. and Weingartner, J. Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease. *Journal of Neuroscience* 1993; **13**: 2540–2550.
58. Niquet, J., Jorquera, I., Ben-Ari, Y. and Represa, A. NCAM immunoreactivity on mossy fibers and reactive astrocytes in the hippocampus of epileptic rats. *Brain Research* 1993; **626**: 106–116.
59. Bendotti, C., Pende, M. and Samanin, R. Expression of GAP-43 in the granule cells of rat hippocampus after seizure induced sprouting of mossy fibres: in situ hybridization and immunocytochemical studies. *European Journal of Neuroscience* 1994; **6**: 509–515.
60. Uchida, Y., Ihara, Y. and Tomonaga, M. Alzheimer's disease brain extract stimulates the survival of cerebral cortical neurons from neonatal rats. *Biochemistry and Biophysics Research Communications* 1988; **150**: 1263–1267.
61. Schwab, M.E. and Thoenen, H. Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. *Journal of Neuroscience* 1985; **5**: 2415–2423.
62. Johnson, A.R., Cook, G.M.W. and Keynes, R.J. *In-vitro* assays for molecules that inhibit growth cone motility during neural development and regeneration. *Neuroprotocols* 1994; **4**: 121–128.
63. Kapfhammer, J.P. and Schwab, M.E. Increased expression of the growth-associated protein GAP-43 in the myelin-free rat spinal cord. *European Journal of Neuroscience* 1994; **6**: 403–411.
64. Chadwick, D.W. New therapeutic horizons in epilepsy. In: *Recent Advances in Clinical Neurology*, Vol. 6 (Ed. C. Kennard). Edinburgh, Churchill-Livingstone, 1990: pp. 209–239.
65. Earle, K.M., Baldwin, M. and Penfield, W. Incisural sclerosis and temporal lobe seizures produced by hippocampal herniation at birth. *Archives of Neurology and Psychiatry (Chicago)* 1953; **69**: 27–42.
66. Gowers, W.R. *Epilepsy and other chronic convulsive disorders: their causes, symptoms and treatment.* London, J. and A. Churchill, 1901: p. 257.
67. Chollet, F. and Weiller, C. Imaging recovery of function following brain injury. *Current Opinion in Neurobiology* 1994; **4**: 226–230.
68. Trimble, M.R. Epilepsy and behaviour. *Epilepsy Research* 1991; **10**: 71–79.